**Cardiorespiratory effects of Gamma-Hydroxybutyric Acid (GHB) during isoflurane**

**anaesthesia in pigs**

**Gamma-Hydroxybutyric Acid effects in pigs**

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**Conflict of interest**

The authors declare no conflict of interest.

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**Authors’ contributions**

CC: study design and execution, data management, data interpretation, manuscript preparation and review.

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GVS: statistical analysis, data interpretation and manuscript review.

CS: manuscript review and data analysis

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**Animal welfare and ethics statement**

The experiment was approved by the ethics committee of the Faculties of Veterinary Medicine and Bioscience Engineering of Ghent University (approval number 086-2021). Care and use of animals were in full compliance with the Belgian (Belgian Royal Decree of May 29, 2013) and European legislation on animal welfare and ethics (2010/63/EU).

**Data availability statement**

The data supporting this study's findings are available from the corresponding author upon reasonable request.

**Abstract**

**Objective** To investigate the haemodynamic effects of gamma-hydroxybutyric acid (GHB) in isoflurane anaesthetised pigs.

**Study Design** Experimental, randomized, non-blinded, cross-over study.

**Animals** A group of six stress-resistant Landrace pigs (approximately 3 months old; three male, three female; bodyweight 39.2 ± 4 kg (mean ± standard deviation).

**Methods** After premedication (midazolam 0.5 mg kg-1 and ketamine 10 mg kg-1 intramuscularly), and induction (propofol 0.25-0.5 mg kg-1 intravenously (IV), anaesthesia was maintained with isoflurane in oxygen, and either GHB 250 mg kg-1 IV or an equal volume of saline was administered (minimum washout period of 1 week). Systolic (SAP), diastolic (DAP), and mean (MAP) arterial pressures, heart rate (HR) and rhythm, respiratory rate (*f*R), were recorded every 5 minutes for 2 hours. Arterial samples were collected for blood gas and pharmacokinetic analyses. Relative changes from baseline were calculated and compared between treatments using a mixed model with time, period, and treatment as variables (*α* 0.05).

**Results** The relative changes from baseline were significantly different between treatments (*p* < 0.001) for SAP (GHB -1.6 ± 10.7; saline -5.9 ± 14.8 mmHg), DAP (GHB +2.9 ± 9.6; saline -6.5 ± 10.7 mmHg) and MAP (GHB +2.2 ± 10.5; saline -5.7 ± 9.6 mmHg). Statistical analysis of secondary outcomes suggested effects on PaO2 (GHB -45.2 ± 29.8 mmHg [-6.03 ± 3.97 kPa]; saline +24.5 ± 32.4 mmHg [+3.27 ± 4.32 kPa]; *p* < 0.001) and PaCO2 (GHB -2 ± 10 mmHg [-0.27 ± 1.33 kPa]; saline -9 ± 8 mmHg [-1.20 ± 1.07 kPa]; *p* < 0.001). The mean maximum blood concentration of GHB was 1171.1 ± 229.3 µg mL-1, with volume of distribution 335.3 ± 68.5 mL kg-1, clearance 77.2 ± 19.12 mL kg-1 hour-1 and elimination half-life 3.10 ± 0.80 hours.

**Conclusions and clinical relevance** GHB did not cause severe physiological side effects and may reduce cardiovascular depression.

***Keywords****:* anaesthesia, cardiorespiratory, gamma-hydroxybutyric acid, pig, sympathomimetic.

**Introduction**

Sodium oxybate, the salt form of gamma-hydroxybutyric acid (GHB), is a naturally occurring neuromodulator and GABA analogue, licensed as a hypertonic injectable solution (Somsanit) for anaesthesia in humans. Oral formulations (Xyrem) are used to treat narcolepsy and as an adjuvant medication to treat the withdrawal symptoms of alcohol use disorder in humans. Its pharmacological effects are mainly GABAergic-dependent (Felmlee et al. 2021)

In humans, GHB causes a dose dependent effect which ranges from relaxation and euphoria, to sleep enhancement, sedation, and anaesthesia (Busardò & Jones, 2015). The latter is characterized by a slow onset, prolonged duration, minimal cardiovascular effects and a lack of analgesic properties (Blumenfeld et al. 1962, Kleinschmidt et al. 1995). After induction of anaesthesia with GHB in 38 patients undergoing advanced cardiac surgery, the mean arterial pressure (MAP) decreased by maximally 10%, without significant changes in heart rate (HR), ventricular filling pressures and cardiac index (Kleinschmidt et al. 1998). More recent reports describe the use of GHB in paediatric patients for cardiac catheterisation (Sauer et al. 2019) and treatment of burn injuries, with potential benefits for wound healing, given its growth hormone stimulating effects (Rousseau et al 2014). In children, doses of 25–50 mg kg-1IV have been considered safe and effective (Rousseau et al. 2012).

 The use of GHB during research studies in animals has been mainly conducted on rodents with fewer studies on larger animals such as cats, dogs and primates. A non-exhaustive table summarizing research on GHB in animals is provided by Van Sassenbroeck (2003). Laborit (1964) reported that in rats, intraperitoneal administration at 500 mg kg-1 induced anaesthesia, while rabbits and dogs required 1000 mg kg-1 intravenously to achieve the same effect. The primary cause of death in case of overdose is respiratory depression. Indeed, mechanically ventilated rabbits tolerated up to 7000 mg kg-1 IV. In dogs, premedication with phenothiazines allowed a 50% a reduction in the effective dose. Anaesthesia onset occurred after 5–10 minutes, with sudden awakening and rapid return to consciousness and motor activity). The duration of action was not mentioned by Laborit (1964).

Other benefits of GHB such as tissue-protective effects have been demonstrated in diverse experimental animal models, indicating its potential therapeutic applicability in conditions involving hypoxia, hypoperfusion, and ischemic injury (Boyd et al. 1990, 1992, 1994, Kolin et al 1993, Ottani et al. 2004, Ysunkaya et al. 2004). Indeed, as a GABA agonist, it prevents oxidative stress, provides cellular energy, and reduces neurotransmission (Mamelak, 1989). However, like other sedatives and anaesthetics, GHB carries a significant risk of abuse (Tay et al. 2022).

In the ongoing quest to optimise anaesthetic protocols, GHB may be useful in a balanced anaesthetic regimen for pigs, which are frequently used for research (cardiovascular, wound healing, organ transplantation) and surgical training (Fleischmann 2023). Given the limited data on the physiological impact of GHB in swine, this study aimed to evaluate the effects of 250 mg kg-1 of GHB on arterial blood pressure in pigs during isoflurane anaesthesia. The secondary aims were to determine the presence of side effects on other routinely measured physiological variables and to assess the pharmacokinetics of IV GHB as a first step in considering the use of this drug for above mentioned indications. Our null hypothesis was that IV GHB would not result in significant differences in hemodynamic and respiratory values during isoflurane anaesthesia compared to saline.

**Materials and methods**

Study design

The experiment was approved by the Ethics Committee of the Faculties of Veterinary Medicine and Bioscience Engineering (approval number 086-2021), University of Ghent. The number of animals enrolled was based on a power calculation [MBESS package (Kelly, 2007) using the R-program (R Core Team, 2017)], targeting a type I error below 5% and a power of 80%. Considering an effect size of 15 mmHg and a standard deviation (SD) of 7.5 mmHg for MAP, six animals were required.

A group of three male and three female stress-resistant Landrace pigs, approximately 3 months old, with a body weight of 30–45 kg and American Society of Anesthesiologists status I (based on physical examination), were included. Pigs were housed together on straw, with *ad libitum* access to a standard pig diet and water for a period of 2 weeks before the study.

Each animal was anaesthetised twice, with at least a 1-week washout period between procedures, receiving either GHB (treatment GHB) or saline (treatment S) in a randomly allocated order, based on a balanced Latin square design by blindly picking the pigs. After anaesthesia induction, a 60 minute period was allowed for instrumentation and stabilisation of end-tidal isoflurane (IsoFlo, Zoetis, Belgium) concentration (FE´Iso)and arterial blood pressure. Then, either GHB (250 mg kg-1, Somsanit, Dr F Köhler Chemie GmBH, Germany) or an equivalent volume of saline (NaCl 0.9%, B Braun, Belgium) was administered IV. The volume of 1.25 mL kg-1 was administered over 5 minutes using a syringe driver (Terufusion Syringe Pump STC-526; Terumo Corporation, Japan) for both saline and GHB. Data acquisition was conducted by two non-blinded observers consistently across all animals, and statistical analysis was performed by a non-blinded author.

Anaesthetic procedure and monitoring

Animals were separated from the group, fasted (with access to water), and weighed 12 hours before each anaesthesia. Ketamine (10 mg kg-1, Ketamidor, Ecuphar NV, Belgium) and midazolam (0.5 mg kg-1, Dormazolam, Dechra Veterinary Products, Belgium) were administered intramuscularly. A catheter (22 gauge, Kruuse, Germany) was aseptically placed in an ear vein and propofol was injected IV, to effect (0.25–0.5 mg kg-1, Propovet, Zoëtis, Belgium) to allow endotracheal intubation. The animals were placed in dorsal recumbency on a surgical table and connected to an anaesthetic machine with an out-of-circuit vaporiser (Dräger AV-1, Drägerwerk, AG, Germany), targeting an FE´Iso of 1.2 %. Isoflurane was vaporized in 60% oxygen, at a fresh-gas-flow of 1.5 L minute-1. Ringer’s lactate solution (Vetivex, Dechra, Belgium) 5 mL kg-1 hour-1 was administered throughout anaesthesia. A second catheter (22 gauge , Kruuse, Germany) was placed in the vein of the contralateral ear. Under ultrasonographic guidance, a catheter (22 SWG, Kruuse, Germany) was aseptically placed in the femoral artery for invasive arterial pressure measurement, using a calibrated pressure transducer (152 cm, 3ML/HR macrodrip, Icumedical, UT USA), positioned at the level of the right atrium, connected to a multi-parameter monitor (Datex-Ohmeda S/5, Finland) and zeroed to atmospheric pressure. Accuracy of the measurement system was verified against a mercury column before use. A square wave test was not performed for each pig. The room temperature was set at 23°C. The isoflurane concentration, systolic (SAP), diastolic (DAP) and mean arterial pressure, HR and heart rhythm using electrocardiography, respiratory rate (*f*R), end-tidal CO2 tension (PECO2), inspired oxygen concentration and oesophageal temperature (T) were monitored with a calibrated, methane-insensitive, multi-parameter monitor (Datex-Ohmeda S/5, Finland). Tidal volume (VT) was measured using a Dräger Volumeter (Drägerwerk, AG, Germany). These variables were recorded every 5 minutes.

To allow evaluation of the effect of GHB on spontaneous ventilation, mechanical ventilation (Drägerwerk, AG, Germany) was only applied if PaCO2 was greater than 80 mmHg or 10.7 kPa in two subsequent measurements, with a VT of 10 mL kg-1 and an *f*R adjusted to maintain PaCO2 < 70 mmHg. If MAP was <60 mmHg for > 10 minutes, a dobutamine (Dobutrexmylan, Cenexi, France) infusion was started at 1 µg kg-1 minute-1 and decreased to 0.5 µg kg-1 minute-1 if MAP increased above 65 mmHg. When base excess (BE) was >10 mmol L-1, saline 5 mL kg-1 hour -1 was infused instead of lactated Ringer’s solution. If spontaneous movements of the head or limbs occurred, 0.25 mg kg -1 propofol was administered IV. The total dose of dobutamine and propofol (used over the 2 hour observation period) was calculated for each pig separately.

, The vaporiser was turned off 2 hours after administration of saline or GHB and the circuit flushed with oxygen. Once the swallowing reflex returned, all catheters and the endotracheal tube were removed, and the pigs placed in lateral recumbency in a straw-filled transport box for recovery. Timepoints for extubation, sternal recumbency and standing were registered.

Blood collection and analysis

To determine GHB concentration, arterial whole blood samples (1 mL) were collected, immediately transferred to Ethylenediaminetetraacetic acid (EDTA) tubes (2 mL, Vacutainer, Company, country) and then stored at 4°C. Before collection of each sample, 1.5 mL of blood was aspirated with a different syringe and discarded. After each sample collection, the catheter was flushed with 3 mL of heparinised (Heparin, LEO, Denmark) saline (NaCl 0.9%, B Braun, Belgium) solution (5 IU mL-1). Blood samples were collected 10 minutes before (baseline) and at 0, 2, 5, 10, 15, 30, 45, 60, 90 and 120 minutes after GHB administration, with timepoint 0 set as the end of the bolus administration.

For blood gas analysis, arterial samples were taken into 2.5 mL heparinised syringes (RapidLyte; Siemens; Ireland) using the collection technique described above and analysed immediately with a calibrated blood gas analyser (ABL 7; Radiometer Medical 128 A/S, Denmark). These samples were obtained 10 minutes before (baseline) and at 15 minute intervals after administration of GHB or saline. The following variables were recorded: arterial pH, partial pressure of oxygen (PaO2) and carbon dioxide (PaCO2), oxygen saturation of haemoglobin (SaO2), base excess (BE), anion gap (angap) and concentrations of bicarbonate (HCO3-), calcium (Ca2+), potassium (K+), sodium (Na+) and chloride (Cl-). The packed cell volume (PCV) was measured by centrifuging capillary tubes (11,181 g, 3 minutes) (Haematocrit 200 centrifuge Hettich, Germany).

GHB concentration via gas chromatography-mass spectrometry (GC-MS)

GHB concentration was determined via GC-MS (Ingels et al. 2010), under ISO17025 accreditation. Within 48 hours, 15 µL of blood was spotted on a 6 mm pre-punched Whatman cellulose filter paper (Merck KGaA, Germany). Samples with expectedly high GHB concentrations were diluted 1/10 or 1/80 with the baseline blood samples (results back-calculated for this dilution afterwards). The blood spots were dried overnight and stored in plastic bags containing desiccant (Merck KGaA) in the dark. On the day of analysis, for each of the calibrators, quality control samples and experimental samples, a dried blood spot was transferred to a glass tube (VWR – Avantor, Belgium), and 10 µL of a 0.025 mg mL-1 GHD-D6 (Cerilliant Corporation, Texas, US) solution was added and dried under nitrogen. The dried blood spots were then derivatised for 10 minutes at 60°C after adding 75 µL of a mixture of heptafluorobutanol (Merck KGaA) and trifluoroacetic acid anhydride (1:2, v:v) (Merck KGaA).
The samples were then dried under nitrogen generated by a N-Gen 64 high performance nitrogen generator (99.0 vol. % N2, Presscon, the Netherlands) and redissolved in 100 µL of ethyl acetate (Chem-Lab NV, Belgium). Then 1 µL was injected on an Agilent 7890A GC system (Agilent, CA, USA) coupled to a 5975 XL mass-selective detector operated by MSD Chemstation software version E.02.02.1431. A 30 \* 0.25 mm i.d. \* 0.25 μm Agilent HP-5-MS column was used. Splitless injections were performed automatically at an injection temperature of 250°C and purge time of 90 seconds, with helium as carrier gas at a constant flow rate (1.3 mL minute-1). The temperature programme started at 65°C for 90 seconds, followed by an increase at 10°C minute-1 to 110°C. The temperature was then raised by 50°C minute-1 to 300°C and held for another 2 minutes. Transfer line and ion source temperatures were set at 280°C and 230°C, respectively. The MS quadrupole temperature was set at 150°C, and an ionisation energy of 70 eV was used. The mass spectrometer operated in selected ion monitoring mode, scanning m/z transitions 227 (quantifier) and 155, 183 and 242 (qualifiers) for GHB and 231 (quantifier) and 161, 189 and 245 (qualifiers) for GHB-D6.

Pharmacokinetic analysis

GHB blood concentration data were analysed using a non-compartmental method (Phoenix, Certara, PA, USA). Pharmacokinetic variables were calculated. These included maximum bloodconcentration at time zero (C0), area under the blood concentration−time curve from T0 to infinity (AUC0−inf), elimination half-life (T1/2el), total body clearance (Cl) and volume of distribution at steady state (Vd). The AUC0−inf was determined using the linear up-log down trapezoidal method and the T1/2el was obtained from the terminal slope of the semilogarithmic concentration–time curve with at least 3-4 time points. Semilogarithmic concentration–time curves were visually inspected.

**Statistical analysis**

The normality of quantitative variables was verified using the Shapiro-Wilk test, skewness and kurtosis evaluation, and visual assessment with boxplots and Q-Q plots. Baseline values were determined by averaging measurements taken at -10 and -5 minutes before administering either GHB or saline for physiological variables. Differences in body weight, baseline values , and recovery times between the two treatments were assessed using a two-sided paired *t-test*. The average amount of propofol and dobutamine usage between treatments was compared using a Wilcoxon Signed Rank Test. Data are presented as mean ± standard deviation for normally distributed variables and as mean and range for non-normally distributed variables.

 Because baseline comparisons of temperature and DAP between treatments revealed significant differences, values were expressed as relative changes from baseline for all variables. These were compared between treatments using mixed model analysis of variance (ANOVA) (lmerTest package, Kuznetsova et al. 2017). A Bonferroni correction was applied to control the familywise error rate, setting the corrected significance threshold at α/20 = 0.0025. Fixed effects included time, period (first or second anaesthesia), and treatment, with pig as random effect. Time was treated as a categorical variable at each time point for blood gas analytes (nine levels) and physiological variables (25 levels). Separate mixed model ANOVAs for each physiological and blood gas variable were conducted. Assumptions of the mixed model ANOVA were evaluated through visual inspection of Q-Q plots for normality of the residuals and residuals versus fitted values plots for homogeneity of variances.

Statistical analyses were conducted using SPSS version 27 (IBM Corp., NY, USA) and R version 4.2.2 (R Core Team, 2017).

**Results**

No animals were excluded from the study. There were no significant differences between treatments for weight (S 39.3 ± 5.4; GHB 39.0 ± 4.8 kg), average total dobutamine use (S 55.8 ± 65.1; GHB 20 ± 49 µg kg -1) or average total propofol top-up dose.(S 0.21 ± 0.29; GHB 0.08 ± 0.20 mg kg -1). Dobutamine was administered to one animal after treatment GHB and to three after saline. Propofol was administered to one animal after treatment GHB and to three after saline. The median (range) FE´Iso was 1.20 (1.17 – 1.23) %.

There was a significant difference at baseline for temperature (GHB 37.8 ± 0.7; S38.6 ± 0.6 °C; *p* = 0.04), and DAP (GHB 49 ± 11; S 55 ± 11 mmHg; *p* = 0.02). In one animal, mechanical ventilation was needed during both treatments (saline and GHB), each time shortly after administration of the treatment drug. The VT data for this animal were excluded.

Results for physiological variables recorded during anaesthesia are presented in Tables 1 and 2. The SAP, DAP and MAP (Fig. 1) increased after GHB and decreased after saline. Although these changes were modest, the relative changes compared to baseline differed significantly between treatments for all these variables; SAP (GHB -1.6 ± 10.7; saline -5.9 ± 14.8 mmHg; *p* < 0.001), DAP (GHB +2.9 ± 9.6; saline -6.5 ± 10.7 mmHg; *p* < 0.001), MAP (GHB +2.2 ± 10.5; saline -5.7 ± 9.64 mmHg; *p* < 0.001). There was a significant effect (*p* < 0.001) of time on SAP and MAP.

With regard to the secondary outcome variables (Tables 1 and 2), the relative changes compared to baseline were significantly different between treatments for Na+ (*p* < 0.001), PaCO2 (*p* = 0.001), PE'CO2 (*p* < 0.001) and PaO2 (*p* < 0.001).

No significant differences were found for the average times ± SD to extubation, sternal recumbency (*p* = 0.205), and standing (*p* = 0.509). The times were 11.3 ± 4.0, 29.8 ± 16.4, and 75.3 ± 19.8 minutes for saline, and 10.3 ± 4.9, 42.2 ± 21.2, and 66.5 ± 20.6 minutes for GHB. All animals were fed 4 hours after recovery.

The mean ± SD C0 was 1171.1 ± 229.3 µg mL-1, Vd 335.3 ± 68.5 mL kg-1, AUC0−inf 1140 ± 249 µg.hour mL-1, Cl 77.2 ± 19.12 mL kg-1 hour-1 and T1/2el 3.10 ± 0.80 hour.

**Discussion**

This study is the first to evaluate the physiological effects of GHB in swine under general anaesthesia. The primary observation was a mild increase in arterial blood pressure after injection of GHB. The main secondary findings were the decrease in PaO2 and a mild elevation in PaCO2 and Na+.

The mild increase in arterial blood pressure induced by GHB, as we observed in this study, has been reported by others (Laborit 1964, Boyd et al., 1992, Van Sassenbroeck et al., 2002; Hicks et al., 2004). Although the hypertonicity of the GHB solution (3.85 Osm L-1) may have contributed to this finding, Van Sassenbroeck et al. (2002) found that GHB (390 mg kg-1 IV) caused a sustained rise in MAP lasting over 2 hours in both control and hypovolaemic rats. This increase was not attributed to sodium content, as an equimolar NaCl solution caused a smaller, shorter-lasting rise. In haemorrhaged rats, GHB (200 mg kg-1 IV) rapidly restored MAP and cardiac output (CO) to pre-haemorrhage levels, whereas NaCl (23%) produced a significant but smaller increase (Boyd et al., 1992). The same study showed that the administration of GHB to hypovolaemic rats induced threefold increases in CO compared to GHB treated normovolemic controls. Similarly, in dogs, GHB (500 mg kg-1 IV) caused a mild, progressive increase in blood pressure (Laborit, 1964). In rodents, IV and intragastric GHB (180 mg kg-1 and 1000 mg kg-1) increased MAP by 24 ± 3 mm Hg over 28 ± 8 minutes and 60 ± 5 mm Hg over 227 ± 37 minutes, respectively (Hicks et al., 2004). However, Laborit (1964) noted a transient decrease in blood pressure in rabbits after intraperitoneal GHB (500-1000 mg kg-1), even after vagotomy. The increase in MAP in rats is likely due to central GABAB receptor activation, enhancing sympathetic activity, though the exact mechanism remains unclear and may involve specific GHB receptor activation (Hicks et al., 2004). Howard and Feigenbaum (1997) also found that GHB increased striatal dopamine production in anaesthetised animals, but inhibited it in awake animals, with variations depending on the route of administration.Changes in dopamine release have been suggested to contributed to dual effects of GHB as a sedative and stimulant (Dudek & Fanelli, 1980). The observed effects on MAP suggest potential use in managing hypotension.

While our study showed that GHB exerted no significant effects on HR, it may have been underpowered for this outcome. The cardiac effects of GHB vary by dose and species. In rats, GHB caused prolonged tachycardia at doses of 560 mg kg-1 and 1000 mg kg-1 IV (Hicks et al., 2004). Conversely, Laborit (1964) consistently observed bradycardia across species, with GHB counteracting phenothiazine-induced tachycardia and hypotension, though specific data were lacking. In humans with GHB intoxication, bradycardia is mainly noted (Blumenfeld et al., 1962; Schep et al., 2012). However, given its cardiovascular stability, GHB has been used for sedation in haemodynamically unstable patients, i.e. during cardiac catheterisation, advanced coronary surgery, and postoperative intensive care (Hunter et al., 1971; Kleinschmidt et al., 1998; Sauer et al., 2019).

After the administration of GHB to the pigs used in our study, an elevation in sodium plasma concentration was detected. This can be explained by the fact that the commercial formulation of GHB is in the salt form, sodium GHB. Each animal received a total dose of 302.86 mg kg-1 of 4-hydroxybutyric acid sodium salt (C4H7NaO3), which was equal to 250 mg kg-1 of GHB and 52.86 mg kg-1 of sodium. This led to a significant increase in plasma sodium levels compared to the isotonic saline treatment, though the levels remained within the reference range for pigs (122–145 mmol L-1) (Perri et al., 2017). For this reason , Somsanit is contraindicated in individuals with kidney disease, arterial hypertension, and hypernatraemia (Somsanit, 2022).

Respiratory depression is often described as one of the side effects of neuroleptics. To evaluate the influence of GHB on ventilation during isoflurane anaesthesia, the pigs in the current study were allowed to breathe spontaneously and a high PECO2 threshold was chosen to initiate mechanical ventilation. Although high PaCO2 levels can affect other variables, GHB only caused a limited increase in PaCO2 compared to saline (Fig.2). However, a significant decrease in arterial oxygen tension was observed after GHB (Fig. 3). To the authors’ knowledge, pulmonary ventilation-perfusion mismatching after GHB has not been reported. Also, VTand *f*R were comparable after both treatments, although the study may have been underpowered for these variables. The reason for a reduction in PaO2 remains unknown, but Hunter et al. (1971) found similar effects of GHB on blood gases in paediatric patients breathing room air for 90 minutes, with a 4 mmHg increase in PaCO2 and a mean PaO2 of 80 mmHg. However, with only minor differences in PaCO2and SaO2 within the range of 99.3–99.9 for both treatments , these findings may have limited clinical relevance in healthy animals. Nevertheless, it is prudent to have equipment for oxygen supplementation and mechanical ventilation available when using this drug, until further studies with larger sample sizes are conducted.

The pharmacological effects of GHB are complex and dose-dependent, showing variability across species (Snead et al. 1976, Godbout and Pivik 1982, Howard and Feigenbaum 1997, Entholzenr 1995). Allometric scaling should be considered, but animals generally require higher doses compared to humans for sedation, making it inadvisable to extrapolate GHB doses between species (Cuypers et al. 2024). For sedation and anaesthesia, doses typically range from 40 to 90 mg kg-1 IV (Somsanit, 2022) in humans and from 150 to 1500 mg kg-1 IV, intragastrically or intraperitoneally in animals (Cuypers et al. 2024). The latter showed sedative effects in pigs starting from 500 mg kg-1 with GHB administered IV as a sole agent. In the present study on pigs under general anaesthesia, where drug interactions may occur, a dose of 250 mg kg-1 was chosen, as an expectedly safe option for examination of its pharmacodynamic effects. Combining therapies in humans, such as GHB with other neuroleptic drugs, has shown a drug-sparing effect, reducing muscle tone and facilitating sedation and anaesthesia (Blumenfeld et al., 1962, Laborit 1964). In dogs, premedication with phenothiazines permitted a decrease in the dose of GHB by 50% (Laborit 1964). In this study, the chosen dose of 250 mg kg-1 was halved compared to Cuypers et al. (2024), resulting in corresponding blood concentrations approximately half those reported with 500 mg kg-1. These levels align with plasma concentrations which, in humans, induced a state described as "medium sleep" (151–293 µg mL-1) to "deep sleep" (244–395 µg mL-1), which might respectively be considered as sedation (i.e., reduced level of consciousness, blinking) and anaesthesia (i.e., no reaction to stimuli; Helrich et al. 1964, Busardò & Jones 2015).

The present study has several limitations. First, despite uniform FE´Iso among all pigs, this may not ensure equivalent depths of anaesthesia, due to potential interindividual pharmacokinetic and pharmacodynamic variability. For this reason, the study was set up as a randomized crossover trial. Secondly, although only objective physiological data were collected, the researchers were not blinded to the treatments, and the collection of such data is not immune to bias. Increased PaCO2 levels are another confounding factor, as modest hypercapnia could increase arterial blood pressure (Battisti-Charbonney, 2011). Although hypercapnia was present after both treatments and the differences between treatments were small, some contribution to the rise in MAP cannot be excluded. It could also be argued that the use of dobutamine and propofol hindered the comparison of arterial pressure between treatments. While no significant differences were found in the total dose of these drugs, the study might have been underpowered for this outcome, warranting further investigation with blinding and a larger sample size. A limitation of pharmacokinetic studies under isoflurane anaesthesia is the potential for anaesthesia-induced changes in drug metabolism and elimination, which may affect outcomes. Nevertheless, these data are valuable for future use of GHB during isoflurane anaesthesia in this species. Further studies are necessary to comprehensively explore the pharmacokinetic profiles of GHB and should consider hypertonic saline as a control when investigating GHB effects. Finally, a sample size calculation was only performed for a change in arterial pressure. Due to the limited literature on the use of GHB in porcine models, predicting expected changes was challenging, making a sample size calculation for all recorded variables difficult. Given the small sample size, the study may have been underpowered for the other variables. The results for these secondary outcomes should therefore be interpreted with caution but can serve as a basis for future research.

**Conclusion**

The IV administration of 250 mg kg-1 of GHB did not cause severe physiological side effects and may reduce cardiovascular depression during isoflurane anaesthesia in pigs Although a mild respiratory depression was seen, as indicated by lower PaO2 and slightly higher PaCO2 values. These findings suggest that GHB can be used safely as an adjunctive drug during isoflurane anaesthesia, but given the limited sample size, further studies are needed to confirm these results.

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**Table 1** Systolic (SAP), mean (MAP) and diastolic (DAP) arterial pressure, heart rate (HR), respiratory rate (fR), end-tidal carbon dioxide (PE ′CO2), tidal volume (VT) and temperature (T) values in six pigs that were administered a single bolus of saline or GHB (250 mg kg-1 IV) during isoflurane anaesthesia (T0 = end of administration). All values were recorded at 5 minute intervals, and the relative change compared to baseline was calculated for all timepoints. Subsequently, the mean relative change was compared between treatments employing a separate mixed model analysis of variance for each variable. Positive values indicate an increase compared to baseline, and negative values indicate a decrease. In addition, values at a few selected time points are displayed as mean ± standard deviation but were not statistically compared between treatments. The SAP, MAP and DAP present the primary outcomes for which the sample size calculation was performed, other results present secondary outcomes. *Significant differences (*p *< 0.0025 after Bonferroni correction) in the mean relative changes are indicated with an \*.* *n* ϯ = 5 for this variable.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variable | Treatment | Baseline | Selected timepoints (minutes) | Mean relative change  | p value |
| T0 | T15 | T30 | T45 | T60 | T75 | T90 | T105 | T120 | (of all timepoints) |  |
| SAP (mmHg)  | GHB | 109 ± 9 | 103±9 | 110±10 | 111±12 | 110±11 | 105±10 | 100±8 | 107±10 | 110±12 | 109±8  | -1.6\* | < 0.001 |
| NaCl | 107±13 | 101±18  | 98±20 | 99±11 | 101±13 | 102±10 | 103±7 | 101±11 | 102±15 | 104± 9  | -5.9\*  |
| MAP (mmHg)  | GHB  | 70±10 | 69±10 | 76±10 | 75±9  | 71±13 | 72±9 | 68±8 | 71±8 | 74±8 | 74±5 | 2.2\*  | < 0.001 |
| NaCl | 73±9 | 66±15  | 66±11 | 66±7  | 67±11 | 70±8 | 66±7 | 67±8 | 70±13 | 67±9  | -5.7\* |
| DAP (mmHg)  | GHB  |  49±11 | 47±11  | 55±12 | 54±11 | 53±12 | 52±14 | 47±10 | 50±9 | 52±7 | 52±6  | 2.9\* | <0.001 |
| NaCl | 55±11 | 47±15  | 48±8 | 47±9  | 48±10 | 51±11 | 45±8 | 48±10 | 53±17 | 48±11 | -6.5\* |
| HR(beats minute -1) | GHB | 112±11 | 109±7  | 118±8 | 114±5 | 117±7 | 118±9 | 122±10 | 124±17 | 130±21 | 132±27 | 10.2 | 0.018 |
| NaCl | 110±10 | 107±10  | 109±13 | 112±10 | 110±11 | 110±11 | 111 ±13 | 110±14 | 109±13 | 111±11 | -0.23 |
| fR(breaths minute-1)  | GHB | 30±4 | 32±4  | 30±3 | 26±8 | 26±8 | 27±9 | 28±9  | 26±9 | 27±8 | 25±10 | -3.6 | 0.182 |
| NaCl | 30±6 | 31±6 | 31±5 | 28±9 | 27±8 | 27±8 | 28±9 |  27±8 | 27± | 28±8 | -1.7 |
| PE ′CO2 (mmHg) | GHB  | 60±14 | 58±13 | 62±15 | 56±5 | 55±5 | 53±8 | 50±8 | 52±10 | 54±10 | 54±10 | -5.4\* | <0.001 |
| NaCl  | 63±11 | 61±10 | 58±11 | 55±8 | 54±11 | 52±10 | 50±10  | 51±9 | 51±11  | 50±9 | -10.2\* |
| V*T* ϯ(mL kg-1)  | GHB  | 4.19±1.12 | 4.70±0.25 | 4.91±0.46 | 4.46±0.34 | 5.05±0.18 | 5.08±0.25 | 5.19±0.83 | 5.00±0.26 | 4.82±0.55 | 5.14±0.63 | 0.8 | 0.815 |
| NaCl  | 4.35±1.06 | 5.03±1.60  | 4.76±1.59 | 5.08±1.51 | 5.59±1.36 | 5.37±1.78 | 5.31±1.45 | 5.26±1.27 | 5.14±1.27 | 5.31±1.39 | 0.9 |
| T (°C)  | GHB  | 38.6±0.6  | 38.6±0.8 | 38.5±0.6 | 38.5±0.7 | 38.5±0.7 | 38.6±0.7 | 38.6±0.8 | 38.7±0.8 | 38.7±0.9 | 38.8±0.9 | -0.0 | 0.006 |
| NaCl  | 37.8±0.7 | 37.8±0.8  | 37.9±0.8 | 37.9±0.8 | 37.9±0.9 | 37.9±0.9 | 38.0±0.9 | 37.9±1 | 38.0±1.0 | 38.0±1.0 | 0.1 |

**Table 2** This table presents secondary outcomes for arterial blood gas analysis, electrolytes and other calculated variables measured in six pigs during isoflurane anaesthesia (T0 = end of administration) following either a single bolus gamma-hydroxybutyric acid (GHB, 250 mg kg-1 IV) or the same volume of saline. Values at selected time points are displayed as mean ± standard deviation. For each time point, the relative change compared to the baseline was calculated, values for each timepoint are not reported in the table, instead the mean relative change compared between treatments is shown in the table.

Note: Significant differences (*p* < 0.0025 after Bonferroni correction) in the mean relative changes are indicated with an \*.Arterial partial pressure of oxygen, PaO2; carbon dioxide, PaCO2; arterial oxygen saturation of haemoglobin, SaO2; packed cell volume, PCV; Base Excess, BE; bicarbonate, HCO3-; calcium Ca2+; potassium, K+; sodium, Na2+; chloride, Cl-; anion gap, angap.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable  | Treatment | Time points in minutes  | Mean relative change | *p* value |
| T-10  | T15 | T30 | T60 | T90 | T120 |
| pH  | GHB | 7.36±0.07 | 7.36±0.08 | 7.38±0.03 | 7.40±0.03 | 7.41±0.03 | 7.40±0.02 | 0.03 | 0.003 |
| NaCl | 7.33±0.07 | 7.35±0.07 | 7.37±0.05  | 7.41±0.04 | 7.41±0.03 | 7.41±0.03 | 0.07 |
| PaO2 (mmHg)kPa | GHB | 224±61 29.9±8.1 | 173±5623.1±7.5 | 166±6722.1±8.9 | 172±71 22.9±9.5 | 184±49 24.5±6.5 | 188±46 25.1±6.1 | -45\* -6\* | 0.001 |
| NaCl | 237±6131.6±8.1 | 245±5832.7±7.7 | 250±43 33.3±5.7 | 256±4334.1±5.7 | 273±4636.4±6.1 | 271±3236.1±4.3 | 25\*3.3\* |
| PaCO2(mmHg)(kPa)  | GHB | 63.5±12.88.5±1.7 | 66.9±15.18.9±2.0 | 64.7±6.3 8.6±0.8 | 61.3±4.88.2±0.6 | 60.6±4.6 8.1±0.6 | 59.6±9.2 7.9±1.2 | -2\* -0.3\* | 0.001 |
| NaCl | 66.8±11.98.9±1.6 | 63.5±12.58.5±1.7 | 61.7±8.18.2±1.1  | 57.9±6.77.7±0.9 | 56.4±5.67.5±0.7 | 56.1±4.67.5±0.6 | -9\*1.2\* |
| SaO2 (%)  | GHB | 99.4±0.4 | 98.9±0.7 | 98.8±0.9 | 99.9±0.6 | 99.3±0.4 | 99.3±0.4 | -0.3 | 0.004 |
| NaCl | 99.8±0.7 | 99.6±0.8 | 99.6±0.2 | 99.7±0.8 | 99.1±0.1 | 99.7±0.4 | 0.2 |
| PCV (%)  | GHB | 28±2 | 27±2 | 26 ±1 | 27±2 | 27±2 | 29±4 | -0.7 | 0.299 |
| NaCl | 29 ±3 | 28±2 | 28±2 | 27±2 | 27±2  | 26±2 | -1.4 |
| BE (mmol L-1) | GHB | 9.6±1.3 | 11±1 | 12±1.6 | 12±2 | 12±2 | 13±2 | 2.3 | 0.637 |
| NaCl | 8.5±1 | 9±0.8 | 9±0.8 | 10±0.8 | 10±0.5 | 10±0.4 | 1.3 |
| HCO3- (mmol L-1) | GHB | 36±1 | 37±2 | 38±1.6 | 38±2 | 38±2 | 39±3 | 2.1 | 0.655 |
| NaCl | 35±1 | 35±1 | 35±0.7 | 36±1 | 35±1 | 36±0.3 | 0.4 |
| Ca2+ (mmol L-1) | GHB | 1.4±0.08 | 1.3±0.06 | 1.3±0.05 | 1.3±0.03 | 1.3±0.04 | 1.2±0.04 | -0.1 | 0.384 |
| NaCl | 1.4±0.09 | 1.4±0.06 | 1.4±0.08  | 1.3±0.03 | 1.3±0.05 | 1.3±0.04 | -0.1 |  |
| K+ (mmol L-1) | GHB | 4.01±0.26 | 3.9±0.2 | 3.86±0.19 | 3.9±0.2 | 3.9±0.2 | 4.0±0.2 | -0.1 | 0.773 |
| NaCl | 4.25±0.66 | 4.2±0.2 | 4.16±0.33 | 4.3±0.3 | 4.3±0.3  | 4.4±0.4 | 0.1 |
| Na2+ (mmol L-1) | GHB | 138±2 | 140±2 | 139±1 | 139±1 | 139±1 | 139±1 | 1.4\* | <0.001 |
| NaCl | 136±3 | 137±2 | 137±2 | 136 ±2 | 136 ±2 | 136±2 | -0.2\* |
| Cl- (mmol L-1) | GHB | 100±3 | 98±2 | 98±3 | 98±1 | 98±3 | 98±1 | -1.7 | 0.789 |
| NaCl | 100±2 | 100±1 | 100±1 | 99±1 | 99±1 | 99±1 | - 0.6 |
| Angap  (mEq L-1) | GHB | 6.2±2.2 | 8.1±2.4 | 6.9±2.5 | 6.6±2.8 | 6.9±2.6 | 6.1±3.2 | 0.8 | 0.141 |
| NaCl | 5.9±2.7 | 6.8±1.9 | 5.6±2.3 | 5.5±2.5 | 6.1±1.7 | 5.7±2.2 | 0.1 |

**Table 3** Systolic (SAP), mean (MAP) and diastolic (DAP) arterial pressure values measured in six pigs during isoflurane anaesthesia (T0 = end of administration) following either a single bolus gamma-hydroxybutyric acid (GHB, 250 mg kg-1 IV) or the same volume of saline. Values at selected time points are displayed as mean ± standard deviation. Positive values indicate an increase compared to baseline, and negative values indicate a decrease. For each time point, the relative change compared to the baseline was calculated, and the mean relative change compared between treatments.

Note: Significant differences (*p* < 0.0025 after Bonferroni correction) in the mean relative changes are indicated with an \*.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variable | Treatment | Baseline | Time in minutes | Mean relative change of all timepoints | p value |
| 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100 | 105 | 110 | 115 | 120 |
| SAP(mmHg) | GHB | 109 ±9 | 103 ±9 | 109 ±10 | 113 ±8 | 110 ± 10 | 103 ± 16 | 107 ±14 | 111 ±12 | 111 ± 10 | 109 ±15 | 110 ±11 | 109 ±11 | 108 ±15 | 105 ±8 | 103 ±8 | 103 ±7 | 100 ±8 | 102 ±8 | 107 ±12 | 107 ±10 | 111 ±11 | 112 ±9 | 110 ±12 | 110 ±8 | 110 ±11 | 109 ±8 | -1.58\* | < 0.001 |
| NaCl | 107 ±14 | 101 ±18 | 90 ±26 | 97 ±16 | 98 ±20 | 98 ±14 | 105 ±10 | 99 ±11 | 100 ±10 | 101 ±10 | 101 ±13 | 107 ±17 | 99 ±15 | 102 ±10 | 103 ±8 | 105 ±8 | 104 ±7 | 104 ±9 | 105 ±9 | 102 ±11 | 106 ±8 | 107 ±7 | 102 ±15 | 103 ±9 | 104 ±9 | 104 ±9 | -5.90\* |
| MAP(mmHg) | GHB | 70 ±10 | 69 ±10 | 73 ±11 | 75 ±9 | 76 ±10 | 71 ±11 | 73 ±10 | 75 ±9 | 75 ±10 | 75 ±12 | 71 ±13 | 74 ±9 | 75 ±12 | 72 ±9 | 71 ±9 | 71 ±8 | 68 ±8 | 68 ±8 | 68 ±7 | 71 ±8 | 74 ±8 | 75 ±7 | 74 ±8 | 73 ±6 | 73 ±7 | 74 ±5 | 2.23\* | < 0.001 |
| NaCl | 73±9 | 66 ±15 | 65 ±16 | 65 ±8 | 67 ±11 | 64 ±8 | 69 ±8 | 66 ±7 | 67 ±7 | 67 ±8 | 67 ±11 | 73 ±8 | 69 ±9 | 70 ±8 | 67 ±8 | 68 ±9 | 66 ±7 | 67 ±7 | 68 ±9 | 67 ±8 | 68 ±9 | 68 ±8 | 70 ±13 | 68 ±8 | 68 ±10 | 67 ±9 | -5.71\* |
| DAP(mmHg) | GHB | 49 ±11 | 47 ±11 | 52 ±12 | 53 ±10 | 55 ±12 | 52 ±12 | 53 ±12 | 54 ±11 | 54 ±12 | 55 ±15 | 53 ±12 | 54 ±12 | 55 ±15 | 52 ±14 | 51 ±12 | 51 ±11 | 47 ±10 | 48 ±10 | 47 ±8 | 50 ±9 | 52 ±9 | 53 ±9 | 52 ±7 | 52 ±7 | 51 ±6 | 52 ±6 | 2.9\* | < 0.001 |
| NaCl | 55 ±11 | 47 ±15 | 50 ±15 | 47 ±6 | 48 ±8 | 45 ±8 | 48 ±7 | 47 ±9 | 48 ±9 | 47 ±9 | 48 ±10 | 55 ±11 | 50 ±13 | 51 ±11 | 47 ±9 | 48 ±11 | 45 ±8 | 47 ±8 | 49 ±10 | 48 ±10 | 48 ±11 | 48 ±10 | 53 ±17 | 48 ±10 | 48 ±11 | 48 ±11 | -6.48\* |

**Figure legend**

**Figure 1.** Change in mean arterial pressure (MAP; in mmHg) (mean ± one standard deviation) *versus* time over a 2-hour period after intravenous administration of GHB (250 mg.kg-1) or an equal volume of saline injected over 5 minutes, ending at timepoint 0, in six pigs. The animals were anaesthetized with 1.2% isoflurane in a mixture of 60% oxygen in air. An overall comparison of relative changes from baseline between treatments demonstrated a significant difference (*p* < 0.001), analysis at separate timepoints was not performed.

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**Figure 2.** Time plot of arterial partial pressure of carbon dioxide (PaCO2) in mmHg (mean ± 1 standard deviation) over a 2 hour period after administration of GHB (250 mg kg-1) or an equal volume of saline over 5 minutes, ending at timepoint 0, in 6 pigs anaesthetized with 1.2% isoflurane in a mixture of 60% oxygen in air. An overall comparison of relative changes from baseline between treatments demonstrated a significant difference (p < 0.001), analysis at separate timepoints was not performed.

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**Figure 3**. Arterial partial pressure of oxygen (PaO2; in mmHg) (mean ± one standard deviation) over a 2 hour period after the intravenous administration of GHB (250 mg kg-1) or an equal volume of saline injected over 5 minutes, ending at timepoint 0, in six pigs. The pigs anaesthetized with 1.2% isoflurane in a mixture of 60% oxygen in air. An overall comparison of relative changes from baseline between treatments demonstrated a significant difference (*p* < 0.001), analysis at separate timepoints was not performed.

